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ON A TREMATODE LARVA ENCYSTED IN A CRAB, *HELICE TRIDENS* (DE HAAN)

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In November, 1915, a considerable number of the encysted larvae of a trematode and some specimens of an infected crab were forwarded for identification by T. Urita, a friend of mine in Kagoshima, with a letter which read as follows: "The liver and the inner surface of carapace of a crab, *Helice tridens* (de Haan), are heavily infested with the egg-like cysts with thick, transparent wall containing a moving worm which seems to be a trematode larva. Some worms creep out. The crabs are found abundantly in the salt-field living in the holes they dig. Seventy to ninety per cent or more among the crabs in the vicinity of Kagoshima City are infected with the cysts."

Afterwards I obtained the crabs of Kagoshima Prefecture on several occasions from the same friend and from H. Yamakado, a student of our academy, and collected them myself on the seashore near Osaka. The crabs are found in various districts, especially in the warm parts of this country. They live in ground near the seashore, such as salt-fields, river mouths, and other coast areas exposed when the tide ebbs, and habitually build burrows in which they live. The crabs are not used as food in Japan proper, but they are said to be eaten in some parts of Korea and Formosa. There is no need to describe the morphological characters of the crab here.

On several occasions I examined two hundred and fifty specimens of the crabs and found about 90 per cent of them infected with the cysts. There is no local difference in frequency of infection between the crabs collected near Osaka and those of Kagoshima. The number of cysts harbored in a crab is variable but generally large, varying from one to several hundreds, though sometimes they are in masses by the thousand. The encysted larvae occur generally in the ovary, on the wall of the stomach, in the hypodermis lining the carapace, and other parts of the body cavity of the host. The ovary (Fig. 1*A*) and hypodermis are most heavily and frequently infested with them. In the liver I have rarely found the cysts, although T. Urita informed me of the liver infection in his letter quoted above. I found occasionally, however, infected pieces of membranous tissues twining round the lobes of the liver, a condition which perhaps would suggest liver infection.

Morphology of the Encysted Larvae.—The encysted larvae found in the crab are oval or rarely spherical in shape, varying more or less in size. Measurements of eight cysts show a range in length from 0.407 mm. to 0.601 mm., and in breadth from 0.366 mm. to 0.510 mm. The average length was 0.521 mm. and breadth, 0.418 mm.

A slight pressure by the cover-glass may alter the dimensions of the cysts; the measurements given above are obtained from those not compressed.

The wall of the cyst is a transparent chitinous membrane from 0.02 to 0.04 mm. thick through which the larva may be readily observed. An actively moving larva in the fully developed cyst is light brown in color with dark spots marking the position of the yolk glands. The worm occupies almost all the space within the cyst with the body bent in various fashion (Figs. 1 *B, C*). In the most common position in the cyst, either the anterior or posterior extremities of the body, or both, coil ventrad, and both lateral margins of the anterior region are also sometimes bent over the ventral surface. Through the wall of the cyst one may recognize the organs of the larva, i. e., oral sucker, alimentary tract including pharynx, long esophagus, intestinal ceca, excretory vesicle, and such genital organs as ovary, yolk glands and testes; but the identification of these organs is complicated by the movements of the body.

The encysted larvae in full development may emerge from the cysts at various times after the latter are removed from the crab and put in distilled water or physiological salt solution. Sometimes this takes place within only ten to thirty minutes after the cysts are removed from the host. Cysts from dead crabs are generally somewhat weakened, in consequence of which the larva may easily emerge from the cyst. Encysted larvae from crabs that have been dead a long time are also dead or so strongly affected as to be at the point of death. It is believed that the action of the putrescent fluids explains the fact that the larvae are generally found out of the cyst and dead. During my study I often found the larvae free and dead after a day or two in culture dishes.

External Feature of the Larva Liberated from the Cyst.—The larva freed from a fresh cyst is actively mobile, changing its shape and size greatly, especially in the anterior region. Figure 2 *B* serves to show the extent of change in the outline of a worm in the contracted state. On the whole, it is concave on the ventral surface and convex on the dorsal; an accurate measurement of length and breadth is very difficult when the worm is alive. The most natural form, however, is to be found in a larva that has recently died a natural death. The larva put between the slide and cover-glass also assumes a form resembling the natural, though slightly exaggerated. The most common form then assumed is an elongated oval, the middle one-half or two thirds of the body length being of nearly uniform breadth. Both extremities taper, the posterior being more obtuse than the anterior end. In seven fixed specimens measured, the length varied from 0.533 to 0.833 mm., and the breadth from 0.283 to 0.325 mm., the average dimensions being 0.701 by 0.301 mm. Two living specimens measured, respectively, 1.0 by 0.50 mm. and 0.922 by 0.43 mm. Four specimens compressed and mounted in potassium acetate varied from 0.9 by 0.466 mm. to 1.18 by 0.56 mm., with an average of 1.083 by 0.515 mm. Five specimens compressed and mounted in Canada balsam varied from 0.75 by 0.35 mm. to 1.07 by 0.50 mm., with an average of 0.918 by 0.438 mm.

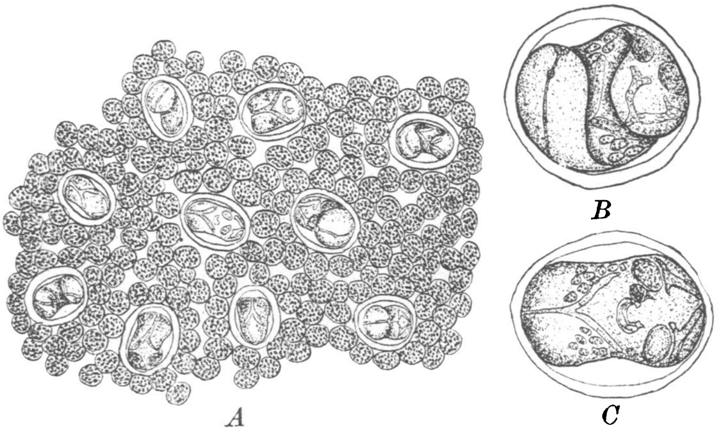

Fig. 1.—*A*, portion of the crab's ovary infested with cysts. × 23. *B, C*, two encysted larvae. × 60.

The entire surface of the body is armed with minute spines which are denser in the anterior region than in the posterior and inconspicuous in mounted specimens. The oral sucker (Fig. 2 *B*) is subterminal and spherical or ovoid in shape; its aperture varies according to the state of contraction of the organ. The ventral sucker (*B*) situated about one-third the body length from the caudal end is oval or spherical and smaller than the oral sucker. In the living specimen it is usually indistinct. The average dimensions of the oral sucker in compressed living specimens were 0.053 by 0.05 mm.; in specimens mounted in potassium acetate, 0.0693 by 0.0625 mm.; in specimens mounted in Canada balsam, 0.052 by 0.055 mm. The average dimensions of the ventral sucker in compressed living specimens were 0.037 by 0.024 mm.; in specimens mounted in potassium acetate, 0.035 by 0.040 mm.

Internal Structure.—The internal structure of the larva (Fig. 2) is easily observed in the living specimens compressed or in the mounted specimens. The larva is remarkable in that almost all the essential genital organs are well developed.

The prepharynx (*P*) is distinct but short and slender, 0.03 to 0.08 mm. long and 0.01 to 0.02 mm. broad. The pharynx (*H*) is subspherical, 0.035 to 0.05 mm. long and 0.025 to 0.048 mm. broad. The esophagus (*E*) is very long and slender, 0.25 to 0.35 mm. long, with the breadth gradually increasing posteriorly to a maximum of 0.015 to 0.027 mm. The intestinal ceca (*C*) are short, running from the posterior end of the esophagus obliquely postero-lateral, so as to form a V-shape, the distal ends being far from the lateral margins of the body. The length of the ceca on both sides is nearly equal in the same individual and slightly variable in the different specimens (0.2 to 0.3 mm. long). The breadth is usually maximum at the middle part of the cecum, but it may be variable according to the condition of its contents.

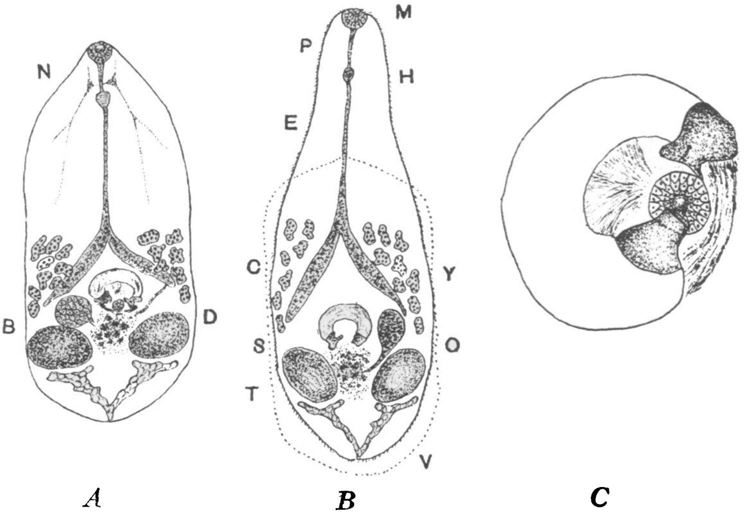

Fig. 2.—*A*, specimen mounted in potassium acetate. × 60. *B*, living larva in motion. × 60. *C*, semilunar organ and ventral sucker. × 280.

The excretory vesicle (*V*) is V- or Y-shaped, and each inner end gives off two branches. The winding and branching canals arise from the anterior end of the vesicle, but their entire course cannot be made out distinctly.

The cerebral ganglion (*N*) surrounding the prepharynx and the lateral nerves from it may be observed in good specimens.

Genital Organs.—Two testes (*T*) lie side by side near the posterior end of the body, just in front of the excretory vesicle. They are oval and subequal with the long axes transverse or obliquely to the axis.

They measure 0.15 to 0.26 mm. in length and 0.09 to 0.16 mm. in breadth. The vas deferens arises from the mesial surface of each testis, but its further course cannot be traced. Just anterior to the ventral sucker there is a peculiar organ (*B*) resembling the cirrus sac. It is semilunar, lying transversely with concave side posterior; each extremity is provided with a chitinous process of form unlike the other. The chitinous process on one side (usually the right) tapers from its wide base and terminates in a smaller single tubular end, while the distal end of the process on the other side is divided into smaller unequal shaped processes (Fig. 2 *C*). The free ends of both chitinous processes face each other across the ventral sucker. The significance of this semilunar organ cannot be determined and no connection with other organs was determined. Before and behind the ventral sucker there are muscle fibers connecting with the semilunar organ.

The ovary (*O*) lies on one side (usually the right) of the median line and in the space bounded by the semilunar organ, the posterior end of the intestinal cecum, and the testis on the same side. It is oval or spherical in shape, being 0.07 to 0.12 mm. long and 0.07 to 0.1 mm. broad. The short oviduct springs from its postero-mesial aspect and runs postero-mediad to a cell-group situated in the space between both testes and the semilunar organ. This probably represents the shell gland and the uterus. Here one may find granules very similar when stained to the yolk granules. The cell-group is deeply stained by carmine.

The yolk glands (*Y*) occupy a small area on the antero-lateral aspect of each cecum. The glands on each side consist of from nine to eleven follicles on the ovarian side and from seven to nine on the other side. These follicles are irregular in form and vary slightly in size; but the maximum diameter varies between 0.055 and 0.08 mm. and the minimum between 0.027 and 0.045 mm. The yolk duct (*D*) from the glands runs postero-mediad to the cell-group mentioned above. The genital aperture could not be detected.

From the characteristics mentioned above it is difficult to determine the adult form corresponding to the encysted larvae. I endeavored to find the matured form by animal experiments. On November 26, 1915, many cysts from the crabs were fed to three guinea-pigs, and on December 14 a number of others to two young cats. These animals died or were killed after several days and were carefully examined for distomes, but in vain. On February 5, 1916, several cysts were eaten by three guinea-pigs, but they did not develop. On April 25 cysts were given to five frogs. After four or five days the frogs were killed and examined for the distomes. In the stomach, intestine, and cloaca larvae were found free from the cysts and dead. Thus far in animal experiments I have not succeeded in obtaining the adult.

These encysted larvae evidently differ from the adult less than in most other cases, because the genital organs are so well developed. The alimentary tract, long esophagus, short intestinal ceca and the position of the yolk glands suggest a relationship to the genus Brachycoelium. Other feeding experiments with the encysted larvae are under way at present and will be described later fully with the microscopical structure of the worm.